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Functionality of Casein Precipitated by Carbon Dioxide[1]

ABSTRACT

The functionality of casein that was precipitated by batch by a novel process using CO_2 was investigated for solubility in water and NaCl, foam stability, overrun, emulsion activity index, and emulsion stability. These properties were compared with those of acidified casein and Na caseinate prepared in the laboratory, commercial acid casein, and commercial Na caseinate and Ca caseinates. The pH of the CO_2 casein was 6.61 in water and 6.51 in 0.1 *M* NaCl; only 65.5% of the casein dissolved in water, which was significantly less than the 97.5% that dissolved in NaCl. The precipitated casein had better foam stability (29 min instead of 1 to 20 min) than did the other caseins, although the percentage overrun was lower (397% compared with 511 to 868%; 15-min whipping time). Emulsion stability (222 min) and emulsion activity index (56 m^2/g) of the precipitated casein were not significantly different from those of caseins prepared in the laboratory but were significantly different from those of commercial caseins. All properties except foam stability were not significantly affected by variations between batches or days. The precipitated casein had functional properties that were significantly different from caseins and caseinates that were produced commercially or in the laboratory.

(**Key words**: casein, carbon dioxide, precipitation, functionality)

Abbreviation key: **EAI** = emulsion activity index, **ES** = emulsion stability, **MFB** = moisture-free basis, **PC** = casein precipitated by CO_2.

INTRODUCTION

Milk proteins have been used extensively as ingredients in other foods because of their high nutritive value and relatively transparent contribution to flavor. Casein, the major protein component of bovine milk, is one of the principal functional food proteins (8). Caseins have exceptional whipping, water-binding, and fat-emulsifying capacities. Caseins are soluble at neutral or alkaline pH; high concentrations are viscous (14). These functional properties have proven valuable for use in a wide variety of products, such as coffee whitener, pasta, and frozen desserts.

Methods of casein manufacture include conventional precipitation by rennet or acidification to pH 4.6, the isoelectric point of casein. Other techniques for the production of casein include salting out with $CaCl_2$ and the addition of a weakly polar solvent, such as ethanol (19). Each of these methods produces a casein with idiosyncratic properties. Recently, Jordan et al. (15) showed that casein could be precipitated from milk by treatment with high pressure CO_2. Tomasula et al. (30) demonstrated the feasibility of carrying out this precipitation in a batch reactor of pilot plant scale and showed that the casein obtained by this method had physical properties similar to, and chemical properties different from, those of commercial caseins. The effect of CO_2 precipitation on the functional properties and subsequent utility in food systems containing casein is not yet established.

Our experiments investigated the functionality of the casein precipitated by CO_2 (**PC**) and compared its functionality with that of conventional commercial caseins and caseinates and with the acidified casein and Na caseinate produced in the laboratory.

MATERIALS AND METHODS

Materials

On each of 3 d, fresh milk was obtained from local dairies, skimmed, and vat pasteurized at 62.8°C for 30 min before being processed. The pasteurized skimmed milk averaged 90.8% water, 0.72% ash, 3.09% protein, and 1277 ppm of Ca^{2+}. The results of the milk analyses were within the standard deviations reported for milk by Jenness (13) and by McBean and Speckmann (18).

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[1]Mention of brand or firm names does not constitute an endorsement by the US Department of Agriculture over others of a similar nature that are not mentioned.
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The casein from 750 g of fresh pasteurized milk was precipitated at 5520 kPa and 38°C for 5 min in a CO_2 batch pressure chamber apparatus as described by Tomasula et al. (30). The pH of the wet casein and whey were measured as soon as possible after the batch reactor was opened. Three batches of PC were prepared from milk each day for a total of nine PC samples. Batches were included in the study only when the PC recovered was at least 65 g (wet weight; sufficient for complete analysis) and the product met microbiological standards for Grade A fluid and dry milks. The PC from each of the nine batches was analyzed wet for the proximate composition, microbial quality, and solubility; the remainder (55 to 90 g) was suspended in about 400 ml of reagent-grade water with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) and freeze-dried. The freeze-dried PC samples were used for the remaining functional and compositional studies.

Acidified casein was prepared from raw skim milk by isoelectric precipitation at pH 4.6 at 30°C with 1 *M* HCl. The curd was separated by filtering through four layers of cheesecloth and washed once with reagent-grade water in an amount equal to 4× the original volume of milk. The washed curd was resuspended using a Polytron homogenizer and stirring in a volume of reagent-grade water equal to the original volume of the milk; the pH was adjusted to 7.0 with 1 *M* NaOH. The solution was filtered through Whatman number 541 paper (Whatman International Ltd., Maidstone, England), and the entire precipitation and washing sequence was repeated. The casein solution was then precipitated again at pH 4.6 with 1 *M* HCl, separated, washed once, resuspended in water, and freeze-dried. Sodium caseinate was prepared in a similar manner except that the 3× precipitated casein was dissolved at pH 7.0 before freeze-drying. The commercial casein and caseinates evaluated were Alacid™ 710, an edible lactic casein; Alanate™ 310, a Ca caseinate; and Alanate™ 130, a granular Na caseinate (all products from New Zealand Milk Products Inc., Santa Rosa, CA). The proximate analyses of these products, as provided by the company, are shown in Table 1.

Proximate Analysis

Moisture (2), ash (2), and Ca^{2+} (21) were determined on the wet samples of PC and fluid milk.

Compositional Analyses

The HPLC analysis of preliminary samples of freeze-dried PC was carried out as described by Strange et al. (28) on a C-8 reversed-phase column, and electrophoretic patterns were determined as described by Van Hekken and Thompson (33) using alkaline urea-PAGE and the PhastSystem™ (Pharmacia, Uppsala, Sweden). Amino acid analysis was completed, and the casein content of all PC samples was calculated (10); commercial and laboratory samples of caseins and caseinates, as well as samples of nonfat dried milk, whey protein concentrate, and total milk protein, were treated similarly.

Bacterial Analyses

Bacteriological analyses for total counts were carried out on the fluid milk and on each of the wet casein samples by the standard plate count or spiral plate count methods (17). Total coliforms and total yeast and mold were analyzed using violet red bile agar and acidified potato-dextrose agar (Difco, Detroit, MI), respectively (17).

Solubility

Solubilities of the commercial and laboratory casein and caseinates and PC samples, as a function of pH, were determined in water and in 0.1 *M* NaCl as described by Strange et al. (27) with the following adaptations. Solutions (0.2%) of dried commercial casein and caseinates and of casein and caseinate prepared in the laboratory were made with water or 0.1 *M* NaCl, pH was adjusted to 7.0 with 1 *M* NaOH

TABLE 1. Proximate analysis of commercial caseins.

Casein[1]	Protein	Moisture	Ash	Fat	Ca^{2+}	Lactose	pH[2]
	(%)						
Alacid™ 710	87.3	9.6	1.8	1.3	0.02	0.1	4.6
Alanate™ 130	83.5	10.1	4.3	1.1	<0.1	0.1	6.7
Alanate™ 310	90.5	4.2	4.1	1.1	1.3	0.1	7.3

[1]New Zealand Milk Products, Inc. (Santa Rosa, CA).

[2]Values are the pH of a 5% solution.

or HCl, and the solutions were stirred overnight. The pH of the solutions were lowered with 1 *M* HCl and sampled every 0.3 pH unit. The protein concentration of each sample was determined after centrifugation by corrected absorbance at 280 nm. The PC samples of known wet casein weight (approximately 0.8 g in 100 g of water or 0.1 *M* NaCl) were titrated with both 1 *M* NaOH to raise the pH to 7.0 and 0.1 and with 1 *M* HCl to lower the pH to 2.0; sampling occurred every 0.3-pH unit. Standard curves were prepared from the corrected absorbance at 280 nm of dilutions of a soluble form for all samples of casein used. The slopes of the standard curves for the PC samples were compared with those for laboratory samples of Na caseinate to calculate a protein concentration for the wet PC.

Foam Formation and Stability

Foam formation and stability were determined using a modification of the method described by Phillips et al. (22) Casein solutions were prepared from the freeze-dried casein by stirring 2.5 g of casein in 40 ml of water overnight. The pH was adjusted to 7 with 1 *M* NaOH, and the volume was adjusted to 50 ml with additional water. The density (ρ) of the protein solution was determined on 10-ml aliquots by comparison of the weight of 10 ml of protein solution with the weight of 10 ml of water. The casein sample was whipped for 5, 10, or 15 min at the number 9 setting of a deluxe mixmaster mixer (Sunbeam Appliance Co., Drowers Grove, IL) equipped with a 1.5-L bowl. Percentage overrun (PO) at each of the three times was determined from the weight of foam in an 87-ml cup (WF) with a 2.9-mm diameter drain hole in the bottom that was closed with a piece of labeling tape during measurements of percentage overrun, which was calculated as follows:

$$\mathrm{PO} = \left[\frac{[(\rho \times 87) - \mathrm{WF}]}{\mathrm{WF}}\right] \times 100.$$

Foam stability was measured on the 15-min foam by opening the drain hole and measuring the time necessary for 50% of the foam weight to drain from the cup. The percentage overrun and foam stability were determined in triplicate for all casein samples described.

Emulsifying Properties

Emulsion activity index (**EAI**) and emulsion stability (**ES**) were measured as described by Van Hekken and Strange (32). Emulsions were prepared from solutions (3:1, vol/vol) of 0.15 *M* NaCl, pH 7, and 0.1% casein to corn oil. The emulsions were formed with the Polytron homogenizer equipped with a PTA 10 shaft using a power setting of 6 for 30 s. Emulsions were heat stressed in a boiling water bath and stirred magnetically. Absorbance at 500 nm (Shimadzu UV-240; Shimadzu, Kyoto, Japan) of a 500-fold emulsion dilution at pH 7 of 0.1% SDS and 0.1 *M* NaCl was measured immediately after emulsion formation and at intervals of 5, 15, or 30 min until absorbance readings were less than one-half of their initial value. Because all casein samples were at pH 7 in 0.15 *M* NaCl, they were assumed to be completely soluble.

$$\mathrm{EAI} = \frac{(2.3)(2)(A_{500\mathrm{nm}})(\mathrm{dilution})}{[(c)(1 - \text{oil volume})(10{,}000)]}$$

where c = grams of casein per milliliter before emulsion, oil volume = 0.25, and $A_{500\mathrm{nm}}$ = initial emulsion absorbance at 500 nm. The EAI reports the square meters of interface stabilized per gram of casein.

The ES was the time (minutes) for the absorbance of the emulsion to decrease 50% and was calculated from the absorbance data using TableCurve Version 2.11 (29). The decrease in absorbance was caused by coalescence and oiling-off, not by creaming.

Carbonate Content

Samples (approximately 100 mg of each) of six of the nine batches of freeze-dried PC and sodium caseinate that were prepared in the laboratory and 1- to 5-mg samples of Na_2CO_3 were sealed into 10-ml vials. To each of these vials, 1 ml of 1 *M* HCl was injected, and the vials were analyzed for CO_2 content by gas chromatography. A 1-ml sample of the headspace of the vials was removed with a 2.5-ml gastight syringe (Hamilton series 1000; Alltech Associates Inc., Deerfield, IL). The gas sample was analyzed by injecting it onto a CTR-1 column (Alltech Associates, Inc.) in a Gow-mac Series 580 gas chromatograph (Gow-mac Inc., Bridgewater, NJ). The He flow was 120 ml/min, and the column was run at ambient temperature. The CTR-1 column can simultaneously separate O_2, N_2, and CO_2. The chromatograms were integrated with an HP 3396A integrator (Hewlett-Packard Co., Avondale, PA). The retention times were as follows: injection peak, 0.14 to 0.2 min; CO_2 peak, 0.45 to 0.60 min; O_2 peak, 1.6 to 2.0 min; and N_2 peak, 2.5 to 3.4 min.

Microscopy

A 1.5-ml sample of 0.8% wet PC in water (used as initial sample for solubility curves) was centrifuged as described by Strange et al. (27), and the pellet was fixed in a solution of 1% glutaraldehyde in 0.1 *M*

imidazole·HCl (pH 6.8), embedded in an epoxy resin mixture, and thin-sectioned for transmission electron microscopy. The photomicrographs were used to determine the size and shape of the insoluble material present in PC.

Statistical Analysis

The ANOVA and other statistical tests, such as Students's *t*, were carried out as described by Steel and Torrie (26).

RESULTS AND DISCUSSION

All of the pasteurized skim milks and the PC made from them were of satisfactory microbiological quality (1, 31) (data not shown). Microbiological analyses were not run on either the commercial or laboratory caseins.

The HPLC and electrophoretic patterns of the PC that were obtained in preliminary studies were similar to the patterns obtained for laboratory Na caseinate. These patterns showed no evidence of preferential precipitation of any of the individual caseins; therefore, no additional experiments were done. Also, we found no detectable levels of whey proteins, which agreed with results reported by Tomasula et al. (30). However, because reversed-phase HPLC does not reliably separate α-LA from α_{S1}-CN (20) and because electrophoresis does not detect very small quantities of whey proteins unless overloaded with the caseins, the amino acid method of Greenberg and Dower (10) was used to analyze the PC for the presence of whey. This method is based on the difference in the Asp and Ala contents of casein and whey proteins. Casein contains 6.31 and 4.12 mol/100 mol of Asp and Ala, respectively, but whey proteins contain 10.21 and 7.48 mol/100 mol, respectively. Calculations using the amino acid contents of the samples showed that the PC were 91.8% ± 3.8 (n = 9) casein; Alanate 310, Alanate 130, and Alacid 710 were 88, 97, and 86% casein, respectively; the laboratory Na caseinate was 98% casein; and the laboratory acid casein was 86% casein. The remainder of the protein was assumed to be whey. These results show that the PC have about the same proportion of whey proteins as found in the commercial and laboratory caseins.

The results of proximate analyses of the PC are shown in Table 2. These results were typical for a wet casein product, except for the Ca^{2+} concentrations, which were high compared with the 0.03% for cottage cheese curd reported by Bassette and Acosta (3) and all three commercial caseins (Table 1). Jordan et al. (15) reported lower Ca^{2+} concentrations for PC than those that were found in this study, but that casein was precipitated at a higher temperature (50°C vs. 38°C). Compared with the Ca^{2+} concentrations found by Tomasula et al. (30), the Ca^{2+} concentrations reported here are within the reported range of their samples. The specific Ca^{2+} concentration of the PC prepared at 38°C and 5520 kPA of Tomasula et al. (30) was significantly lower than all other reported Ca^{2+} concentrations except for the PC prepared at 38°C and 1440 kPA, but Tomasula et al. (30) also reported variable Ca^{2+} concentrations. The amount of Ca^{2+} found in our samples [1.9% moisture-free basis (**MFB**)] represented about 54% of the amount of Ca^{2+} found in casein milk micelles (3.5% MFB) as calculated from data presented by Holt et al. (12).

TABLE 2. Proximate analysis of casein precipitated by CO_2.

	d 1		d 2		d 3	
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
Protein,[1] %	94.20	19.50	94.40	10.30	97.40	2.30
Moisture, %	80.50	3.20	81.00	1.50	78.00	1.30
Ash,[2] %	6.46[b]	0.74	4.47[a]	0.29	6.86[b]	0.46
Ca^{2+},[2] %	1.96	0.14	1.82	0.17	1.95	0.16

[a,b]Means within a row with no common superscript letter differ ($P < 0.01$).

[1]Percentage of protein calculated from spectrophotometric data, moisture-free basis.

[2]Moisture-free basis.

The pH of the PC from the batch reactor was 5.63 ± 0.08 (n = 9), and the pH of the whey was 6.23 ± 0.06 (n = 9). The pH of the PC was lower than the pH 5.8 of the reaction reported by Tomasula et al. (30), but subsequent experiments have shown a pH of 5.6 when the position of the pH probe in the whey removal line was slightly altered. However, Gevaidan et al. (9) reported a pH of 4.75 at 2000 kPa of CO_2 pressure but observed no casein precipitation. Tomasula et al. (30) carried out the precipitation process at temperatures from 32 to 60°C and pressures of 2760 to 5520 kPa. Gevaudan et al. (9) studied the process at 5°C and 500 to 1500 kPA. Tomasula et al. (30) reported a pH of 5.6 during the precipitation process at 38°C at 5520 kPa. At pH 5.6, the colloidal $CaPO_4$ is partially dissolved, which destabilized the casein micelle; an increase in the Ca^{2+} concentration in the serum phase (24 m*M* at pH of 5, 20 m*M* at pH of 5.5, and 12 m*M* at pH of 6.0) (16) further destabilized the casein. Van Hekken and Strange (32) showed that the Ca^{2+} concentration required to precipitate 80% of whole casein at pH 7 was 15 m*M*, and Dalgleish and Parker (5) reported that the Ca^{2+} concentration needed to

initiate casein precipitation tended to decrease as pH decreased and temperature increased. High pressure may also contribute to the destabilization of the casein micelle. Schmidt and Buchheim (25) showed distortion of the micelle after treatment at 10 MPa, and Desorby-Banon et al. (6) showed that milk, after pressurization at 200 MPa, began to clot at a significantly higher pH (5.3 vs. 5.0) than did unpressurized milk. The mechanism for the precipitation of casein during treatment with high pressure CO_2 is probably a combination of effects including acid precipitation, Ca^{2+} precipitation, high pressure distortion of micelles, and other destabilizing physicochemical effects from the presence of high levels of CO_2 dissolved in milk.

At the pH of the PC (5.6), Le Graet and Brulé (16) reported that casein retained about 1.8% Ca (MFB), and Brulé and Fauquant (4) reported that casein retains about 1.45% Ca (MFB). Of the Ca associated with the PC, about 50% could have been bound to the casein (12), but the remainder was in nonnative form because the casein had completely aggregated and no longer contained colloidal $CaPO_4$. The most likely form would be $Ca_3(PO_4)_2$, not $CaCO_3$ or $Ca(HCO_3)_2$. Gas chromatography of the headspace of acid-treated freeze-dried PC showed 0.08% CO_2, which corresponds to <0.02% CO_3 for the sample size measured.

The PC contained about 1.5 times the Ca^{2+} content of commercial Ca caseinate, Alanate™ 310, which has a Ca^{2+} content of 1.3% (Table 1). Commercial Ca caseinate is manufactured by suspension of washed acidified casein curd in water, followed by titration to the desired pH by addition of $Ca(OH)_2$ dispersed in water; at pH 6.7, the Ca caseinate contained 1.33% Ca (MFB) (24). At least one-half of the Ca^{2+} content of the PC is from the Ca caseinate complex that is native to milk, rather than from added $Ca(OH)_2$.

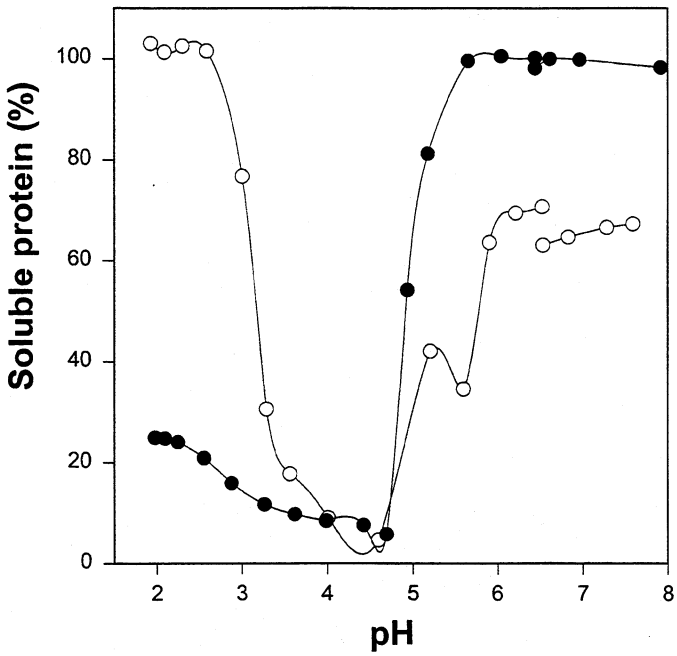

Figure 1. Solubility of CO_2-precipitated casein in reagent-grade water (○) and in 0.1 *M* NaCl (●) as a function of pH.

The solubility of the PC was determined at various pH in water and in 0.1 *M* NaCl. Typical solubility curves are shown in Figure 1. When dispersed in water (approximately 0.8 g/100 g of water), the PC had a pH of 6.61 and, when dispersed in 0.1 *M* NaCl, the PC had a pH of 6.51. There was no difference ($P > 0.05$) in the pH of the two solutions. The wet PC was stirred in reagent-grade water or 0.1 *M* NaCl for at

TABLE 3. The percentage of overrun for caseins after 5, 10, and 15 min of whipping and the time needed for 50% collapse of the foam.

	CO_2-Precipitated casein (n = 27)		Acidified casein (n = 3)		Na Caseinate (n = 3)		Alacid™ 710[1] Lactic casein (n = 3)		Alanate™ 130[1] Na Caseinate (n = 3)		Alanate™ 310[1] Ca Caseinate (n = 3)	
	(% of overrun)											
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
Whipping time												
5 min	270^{d}	41	278^{d}	7.1	487^{c}	17	545^{c}	36	634^{b}	3.1	773^{a}	12
10 min	341^{d}	78	345^{d}	19	557^{c}	19	581^{c}	37	686^{b}	5	835^{a}	11
15 min	397^{f}	92	511^{e}	6	645^{c}	6	586^{d}	29	727^{b}	6	870^{a}	13
	(min)											
Time for 50% foam collapse	28.5^{a}	16[2]	20.2^{b}	1.6	1.11^{d}	0.22	17.9^{b}	1.7	10.2^{c}	1.1	15.8^{b}	2.1

[a,b,c,d,e,f]Means within a row with no common superscript differ ($P < 0.05$).

[1]New Zealand Milk Products, Inc. (Santa Rosa, CA).

[2]The ANOVA of the data shows variation between batches ($P < 0.01$) but not between days.

TABLE 4. Emulsion activity index (EAI) and emulsion stability (ES) of CO_2-precipitated caseins and standard and commercial caseins.

Casein	EAI			ES		
	— (m^2/g) —		(n)	— (min) —		(n)
	$\bar{X}$	SE		$\bar{X}$	SE	
CO_2-Precipitated casein	55.8[b]	5.1	72	222[a]	27	9
Acidified casein	57.3[b]	2.1	8	167[a]	91	2
Na Caseinate	59.6[b]	1.9	8	209[a]	6	2
Alacid™ 710[1]	25.2[c]	2.5	8	14[b]	0.05	2
Alanate™ 130	70.0[a]	2.1	8	147[b]	8.5	2
Alanate™ 310	60.3[b]	1.5	8	19[b]	3.1	2

[a,b,c]Means within the same column with no common superscript differ ($P < 0.01$).

[1]New Zealand and Milk Products, Inc. (Santa Rosa, CA).

least 1 h before any solubility measurement was made. However, the NaCl solution dissolved more protein ($P < 0.01$) than did water (97.5 ± 5% vs. 65.5 ± 8%), which suggested that the high Ca^{2+} content of the PC might have been responsible for the observed difference in solubility between water and NaCl. The solubility of $Ca_3(PO_4)_2$ increased as the ionic strength of the solution increased (23), and casein coprecipitated with $Ca_3(PO_4)_2$ would dissolve when the solubility of $Ca_3(PO_4)_2$ increased. For casein with bound Ca^{2+}, the Na^+ would replace casein-bound Ca^{2+}, also increasing solubility (27).

As the pH was lowered, the PC in water became less soluble until isoelectric pH 4.6 was attained. Below pH 4.6, the solubility of the PC increased until about pH 2.8 when PC was completely soluble. When the pH of each of these solutions was returned to neutrality, the PC was completely soluble. The casein aggregates that were initially insoluble in water were destroyed by lowering the pH, probably because of the presence of small casein aggregates held together by precipitated $Ca_3(PO_4)_2$ that did not reform when the pH was raised.

Transmission electron micrographs of the insoluble aggregates (data not presented) showed irregularly shaped particles that were approximately 0.15 to 0.3 μm in length and 0.05 to 0.1 μm in width, which were possibly fragments of an acidified casein gel. The particles were easily sedimented.

The PC dissolved in 0.1 *M* NaCl exhibited behavior that was typical of a soluble caseinate (27). At pH conditions above the isoelectric point, casein was completely soluble; at pH conditions close to the isoelectric point, casein was insoluble; at pH conditions below the isoelectric point, about 25% of the protein was soluble. The solubility curve of Alacid™ 710 in water showed, at a pH <3, a 25% decrease in solubility compared with that of the other caseins and almost complete insolubility at pH between 3.5 and 6.9 compared with an insoluble pH range of 4 to 5 for the other caseins. At pH 7, the Alacid™ 710 was soluble. The solubility curve of Alacid™ 710 in 0.1 *M* NaCl also showed that solubility at a pH <6 was lower than that of the other caseins. The solubility of the laboratory acidified casein and caseinate and of the other commercial caseins in water and in 0.1 *M* NaCl were typical of a soluble caseinate.

The percentage of overrun and foam stability of the PC and of the commercial and laboratory caseins are listed in Table 3. The percentage overrun of the PC showed no variation ($P > 0.05$) between batches or between days; the foam stability varied ($P < 0.01$) between batches but not between days. The foam stability was increased in the order batch 1, batch 2, and batch 3 for each of the 3 d of processing. This variation may be an artifact of the foam stability assay order because no attempt at sample randomization was made.

The percentage overrun of the PC was not different ($P > 0.05$) from that of the acidified casein prepared in the laboratory when whipped for 5 or 10 min (Table 3). The percentage overruns of the Na caseinate prepared in the laboratory and the commercial caseins were higher ($P < 0.01$) (Table 3) than those of the PC, but the foam stability of the PC was significantly better. The percentage overrun increased as whipping time increased for all of the caseins; these increases in percentage overrun have been attributed to the resistance of casein to denaturation and aggregation (22). Increases in foamability and stability have been noted for whey protein isolate when foamed in the presence of divalent cations (34). The PC has a higher Ca^{2+} content than any of the commercial caseins; a 5% solution has about 20 m*M* Ca^{2+}. Because casein is more sensitive to the presence of Ca^{2+} than are whey proteins, the higher Ca^{2+} content of PC might have been responsible for the increased foam stability. Increased foam stability may

also be related to the high foam density (reflected in lower percentage overrun), which encouraged increased drainage stability (11). The lower percentage overrun is due to the lower solubility of PC in water, which resulted in less protein available for forming the air-water interfaces of a foam.

The EAI showed no variation ($P > 0.05$) between days (Table 4). The EAI of the PC was significantly higher than that of a commercial lactic acid casein (Alacid™ 710) but significantly lower than that of a commercial sodium caseinate (Alanate™ 130). Because all of the caseins studied were completely soluble in NaCl solutions at pH 7, these differences are not due to solubility (11), and, although Ca^{2+} can cause alterations in the emulsion properties of casein (7), the concentrations of Ca^{2+} that were present in a 0.1% PC solution (0.5 m*M*) were much lower than the 7 m*M* needed to effect emulsions (7).

Emulsion stability of the PC was numerically greater than those of any of the other caseins tested and greater ($P < 0.01$) than any of the commercial caseins. These differences occurred despite adjustment to pH 7 in all cases. Examination of the data in Table 4 show two groupings in the ES data. The differences observed may be because commercial samples were spray-dried rather than freeze-dried.

CONCLUSIONS

The PC were partly insoluble at pH 7, which could have affected the functional properties, as shown by comparison with casein prepared either commercially or in the laboratory. The solubility behavior of the PC suggests that the Ca^{2+} is bound differently from Ca caseinate, especially because the Ca^{2+} content of the PC is greater and no Ca^{2+} was added. The PC produced the most stable foams and emulsions but did not have the greatest whippability or the highest EAI. The batch process for gas precipitation produced caseins that had functional properties that were different from those of casein produced commercially or in the laboratory and were consistent among the batches (except for foam stability) and days.

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